Potency of Melaleuca bracteata and Ocimum sp. Leaf Extracts as Fruit Fly (Bactrocera dorsalis complex) Attractants in Guava and Star Fruit Orchards in Bogor, West Java, Indonesia

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Potency of *Melaleuca bracteata* and *Ocimum* sp. Leaf Extracts as Fruit Fly (*Bactrocera dorsalis* complex) Attractants in Guava and Star Fruit Orchards in Bogor, West Java, Indonesia

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Fruit flies such as the *Bactrocera dorsalis* complex (Diptera: Tephritidae) are serious horticultural pests. In Indonesia—especially in Bogor, West Java—fruit flies are major pests on guava and star fruit, both valuable crops. Synthetic insecticides are popular for fruit fly control. However, their inappropriate use can negatively affect the environment and humans. Methyl eugenol extracted from the leaves of plants such as *Melaleuca bracteata* and *Ocimum* sp. as a male fruit fly attractant is an environmentally friendly and consumer-safe alternative for control. We determined the potency of leaf extracts as fruit fly attractants by observing their effectiveness against the most destructive fruit flies (*B. dorsalis* complex) in guava and star fruit orchard, therefore identification to both species is needed. Seven attractants were tested for trapping fruit flies: essential oils of *M. bracteata* and *Ocimum* sp., along with each residual distillation water, mash extracts of each species, and commercial methyl eugenol as a control. Both essential oils showed high potential as fruit fly attractants. For more than 2 weeks they attracted male fruit flies in guava and star fruit orchards as effectively as commercial methyl eugenol. Distillation water and mash extracts from the leaves of both plants lasted for a maximum of 6 days. In a guava orchard, *M. bracteata* and *Ocimum* sp. essential oils and the control trapped 78.75, 77.5, and 88.75 fruit flies, respectively, over 2 weeks, and in a star fruit orchard they trapped 35.75, 38.75, and 40.50, respectively. Capture rates using distillation water and mash extracts from both plants were significantly lower than those using essential oils in both orchards. In the guava orchard 57.14% of the fruit flies trapped were *B. papayae* and 42.85% *B. carambolae* (n=70); in the star fruit orchard, 45.71% were *B. papayae* and 54.28% were *B. carambolae* (n=70).

Key words: Fruit flies, Leaf extracts, *Melaleuca bracteata*, *Ocimum* sp.

Introduction

Fruit flies of the *Bactrocera dorsalis* complex (Diptera: Tephritidae) are major pests of various horticultural commodities in many countries. In Southeast Asia, fruit flies include considerably more pest species than in countries in other regions and cause more economic problems. Losses due to fruit flies can be categorized as loss of crop production, decreased export trade, and increase pressure and costs of quarantine services (Drew and Romig, 1997).

In Indonesia, and especially in West Java, fruit flies are a major pest on guava and star fruit, both of which have high economic value. The highest production of guava fruit is in West Java with 157,030 tonnes/year. Star fruit production in West Java is the second largest in Indonesia at 16,727 tonnes/year (Central Board of Statistics of the Republic of Indonesia, 2011). Guava and star fruit orchards are available in Bogor, West Java, where the popular cultivars are Getas merah for

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guava and Dewa for star fruit (The Provincial Government of West Java, 2012).

There are several ways to control fruit flies, such as insecticide cover sprays, bait sprays, the sterile insect technique, physical control, and biological control (Vijaysegaran, 1997). In Indonesia, application of synthetic insecticides is a popular measure for controlling fruit flies. However, inappropriate use of insecticides has negative impacts on the environment and human health. There is an environmentally friendly alternative for fruit fly control that is safe for consumers—specifically, the use of the plant extract methyl eugenol as an attractant for trapping male fruit flies (Kardinan, 2003).

Methyl eugenol has an important role in the mating success of *B. dorsalis* complex. Male fruit flies in the *B. dorsalis* complex consume methyl eugenol to produce pheromone to attract females of the *B. dorsalis* complex (Nishida *et al*., 1988). Male fruit flies are able to detect the scent of methyl eugenol within 1−3 km, depending on the wind direction (Kardinan, 2003). In nature, methyl eugenol is produced by several crops, including *Melaleuca bracteata* and *Ocimum* sp.

M. bracteata or Black Tea Tree and *Ocimum* sp. or Holy Basil or Angelica Herbs are easy to cultivate in Indonesia. The essential oil of *M. bracteata* contains up to 76% methyl eugenol (Wikardi *et al*., 1993) and that of *Ocimum* sp. contains up to 73.6% (Kardinan *et al*., 2009). Methyl eugenol can be obtained from these plants by several processes, such as mashing of the plant leaves, resulting in a mash extract, and by distillation, which results in an essential oil and distillation water.

The potency of leaf extracts for attracting fruit flies can be determined by observing the duration of their effectiveness as well as the number and species of fruit flies trapped.

Materials and Methods

Experiments were conducted in guava and star fruit orchards in Bogor, West Java, Indonesia, in July and August 2012. All extractions were conducted in the laboratory of the Indonesian Spices and Medicinal Crops Research Institute (IMACRI; http://balittro.litbang.deptan.go.id/eng/), Bogor, West Java, Indonesia. Fruit flies were identified at Bogor Agricultural University (IPB) Bogor, West Java, Indonesia.

Extraction

Essential oil and distillation water

Melaleuca bracteata and *Ocimum* sp. leaves were collected from IMACRI. Essential oils and distillation water were made by a distillation process. 10 Kg of *M. bracteata* and *Ocimum* sp. leaf (respectively) are processed to distillator with 2 L of water. From the *M. bracteata* and *Ocimum* sp., obtain about 150 mL and 100 mL of essential oil respectively and the residual water is used as distillation water.

Mash extracts

Mash extracts were made by crushing 1 kg of *M. bracteata* or *Ocimum* sp. leaves in 1 L of water (i.e., 1:1 [wt:rol] leaves:water).

Fruit fly traps

Fruit fly traps were made from plastic mineral-water bottles. There are one hole (on very side of the bottle (total＝3 hole) were made. The attractant (leaf extract) was dropped on a cotton ball hanging inside the trap. Except for mash extract, the attractant is mixed with 15 mL of water that is placed in the bottom of bottle. The trap also included 30 mL of water to kill the fruit flies. Three types of leaf extract were tested as fruit fly attractants in this experiment, and each had a different dose: essential oil, 1 mL/trap; distillation water, 4 mL/trap; and mash extract, 15 mL/trap. Commercial methyl eugenol was used as a control, as much as 1 mL/trap. Fruit fly traps were hung on guava or star fruit trees about 2 m above the soil surface.

Fruit fly effective capture duration and number of flies trapped

The study was conducted in Bogor, West Java, Indonesia, in guava and star fruit orchards with total areas of 8000 m^2 and 6000 m^2, respectively. The experiment used a completely randomized design, with seven treatments with four replicates each; 28 traps were placed in each orchard. The traps were hung on guava or star fruit trees about 2 m above the soil surface.

The distance between traps was 3 to 5 m. Observations included the number of flies caught per day per trap and the total number of flies caught over 2 weeks in all trap. Observations were conducted over 2 weeks at 2-day intervals (seven observations). The data obtained were tested by using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test.

Fruit fly identification

We identified the species of fruit flies caught by using each treatment (extract). A total of 10 flies were taken randomly from each treatment in each orchard (70 specimens per orchard). The *B. dorsalis* complex consists of 52 sibling species with similar morphology (Drew and Hancock, 1994). It is still difficult to distinguish five species in the *B. dorsalis* complex (*B. philippinensis*, *B. occipitalis*, *B. carambolae*, *B. papayae*, and *B. dorsalis*); these five species are major pests in Asia (Iwahashi, 2001).

Aedeagal length can be a good feature for distinguishing sympatric species of the *B. dorsalis* complex. Iwahashi (1999) distinguished *B. papayae* and *B. carambolae* on the basis of aedeagal length and the band types on the abdomen (transverse black band on tergite 4). *Bactrocera papayae* has an aedeagal length range of 2.66 to 3.34 mm; for *B. carambolae* the range is 2.39 to 2.68 mm.

Fruit fly identification followed the key of the Centre for Agricultural Bioscience International (CABI) (White and Hancock, 1997), with further identification by measuring the length of the aedeagus and observing the dorsal abdominal pattern in accordance with the method of Iwahashi (1999). Measurements of the aedeagus were performed by dissecting the end of the male fruit fly abdomen and then mounting the aedeagus on a glass microscope slide with double-sided tape. The aedeagus was then measured under an Olympus microscope with DPSW 20 image analysis software.

Results and Discussion

Duration of trapping

The essential oils from *M. bracteata* and *Ocimum* sp. had the longest period of effectiveness for trapping fruit flies. In guava and star fruit orchards both essential oils lasted for more than 2 weeks (Figure 1). The essential oil of *Ocimum* sp. lasted for up to 5 weeks in mango orchards (Kardinan e*t al*., 2009). The distillation water and mash extract from *M. bracteata* and *Ocimum* sp. lasted for up to 1 week in both orchards. The essential oil of *Ocimum* sp., contains 73.5% methyl eugenol, whereas *Ocimum* sp. distillation water contains only 0.43%. Thus the duration of trapping effectiveness was affected by the percentage of methyl eugenol in the extract (Kardinan e*t al*., 2009).

Duration of trapping of fruit flies is also determined by the presence of methyl eugenol in the air. The presence of methyl eugenol may decline depending on environment factors, greatest affects from temperature and humidity causing evaporation (Shaver and Bull, 1980). Methyl eugenol mixed with distilled water and mash extract easily evapoarates because both extract containers are water base. In the atmosphere, methyl eugenol is not affected by photolysis from the UV spectrum due to the lack of absorption by the environmental UV spectrum (Meylan and Howard 1993). The empirical data from a biodegradation study of methyl eugenol showed approximately 90% ultimate biodegradation over 28 days using an activated sludge inoculum (Toxicology Data Network, 2012).

The results from the guava orchard showed that the essential oils of *M. bracteata* and *Ocimum* sp. trapped a significantly greater number of fruit flies than did the distillation water and mash extracts of both plants ($P < 0.05$; Table 1). There was also a significant difference between the number of fruit flies (over the 2-week study period) trapped by commercial methyl eugenol (89 per trap) and the essential oils of *M. bracteata* and *Ocimum* sp. (79 and 77, respectively). Similarly, in star fruit orchards the essential oils of *M. bracteaca* and *Ocimum* sp. trapped significantly more flies than did distillation water and mash extracts of both plants (Table 1). However, there was no significant difference between the number of flies trapped by the control (40; commercial methyl eugenol) and the essential oils of *M. bracteaca* (36) and *Ocimum* sp. (39).

As might be expected, the number of fruit flies in the field is affected by biotic and abiotic factors. Abiotic factors such as the amount of rainfall and number of rainy days have a positive and highly significant correlation with fruit fly populations (Hasyim *et al*., 2008). Our experiments were conducted during the dry season, when there is low-intensity rainfall of short duration. Thus the fruit fly population in the field was relatively small.

There were different total numbers of fruit flies trapped in guava orchards (1098) and star fruit orchards (583). It is possible this reflects a difference between orchards in the application of synthetic insecticides. Information provided by farmers indicates that, in the star fruit orchards, three commercial synthetic insecticides are applied four times a month. Insecticide application starts as soon as the star fruit flowers appear. In contrast, a single commercial insecticide is applied once a month in guava orchards.

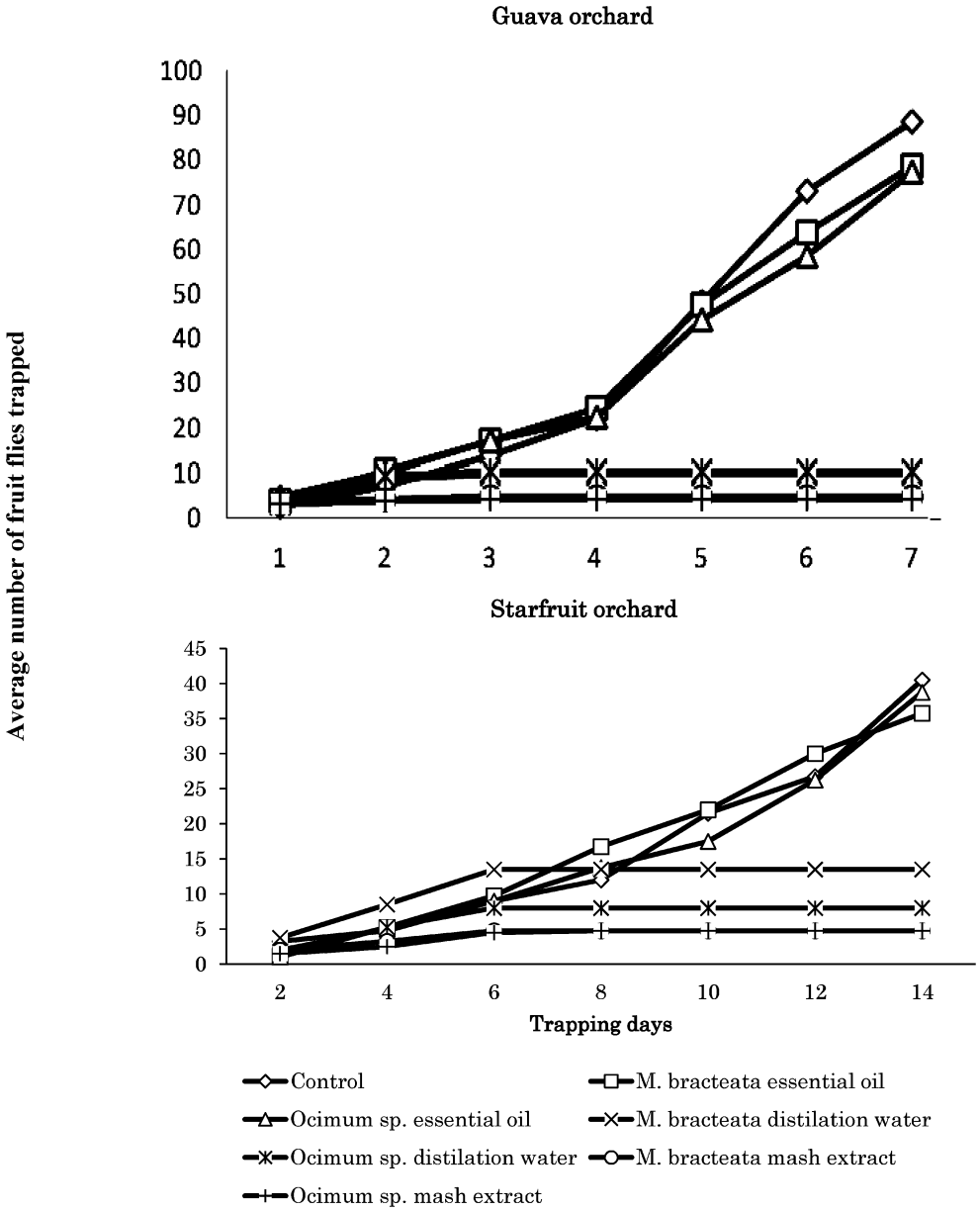

Fig. 1. Effective duration of *M. bracteata* and *Ocimum* sp. leaf extracts as fruit fly attractants in guava and star fruit orchards. Averages are based on the cumulative number of male fruit flies captured; fruit flies in traps were counted every 2 days. Each attractant was used in four replicate traps in each orchard.

Bactrocera dorsalis complex identification

Two species of fruit fly were caught by the extracts in both orchards: *B. carambolae* and *B. papayae*. The aedeagal length range of specimens is presented in Table 2. In the guava orchard, 57.14% of the flies trapped were *B. papayae* and 42.85% were *B. carambolae* (n=70). In the star fruit orchard, 45.71% were *B. papayae* and 54.28% were *B. carambolae* (n=70).

B. carambolae is one of the primary pests on star fruit and guava fruit in Indonesia. *B. papayae* is a primary pest on many horticultural commodities in Indonesia such as mango, chili, orange, guava, and star fruit. *B. papayae* has the widest range of hosts, with 51 host plant families (Clarke *et al*. 2005).

Table 1. Average (from four replication) number of fruit flies caught per treatment over 2 weeks in guava and star fruit orchards in Bogor, West Java, Indonesia

Orchard	Average number of fruit flies per treatment[a]						
	Commercial methyl eugenol	*M. bracteata*			*Ocimum* sp.		
		Dw	Eo	Me	Dw	Eo	Me
Guava	88.75 (a)	9.75 (c)	78.75 (b)	5.00 (d)	10.50 (c)	77.5 (b)	4.25 (d)
Star fruit	40.50 (a)	8.00 (b)	35.75 (a)	4.50 (c)	13.50 (b)	38.75 (a)	4.75 (c)

[a] Numbers followed by the same letters within each orchard type are not significantly different ($P>0.05$; Duncan's Multiple Range Test). Dw, distillation water; Me, mash extract; Eo, essential oil.

Table 2. Distribution of aedeagal lengths and species of fruit fly specimens trapped in guava and star fruit orchards in Bogor, West Java, Indonesia

Attractant[a]	Number of fruit flies in each aedeagal size range and species							
	Guava				Star fruit			
	Aedeagal length range (mm)[b]		Species[c]		Aedeagal length range (mm)		Species	
	2.34–2.68	2.66–3.34	*B. carambolae*	*B. papayae*	2.34–2.68	2.66–3.34	*B. carambolae*	*B. papayae*
CME	5	5	5	5	7	3	6	4
M.Dw	5	5	4	6	7	3	7	3
M.Eo	5	5	4	6	5	5	6	4
M.Me	3	7	4	6	4	6	3	7
O.Dw	6	4	5	5	6	4	6	4
O.Eo	4	6	4	6	5	5	5	5
O.Me	5	5	4	6	6	4	5	5
Total			30	40			38	32
Percentage			42.85%	57.14%			54.28%	45.71%

[a] CME, commercial methyl eugenol; M., *M. bracteata*; O., *Ocimum* sp.; Dw, distillation water; Eo, essential oil; Me, mash extract. [b] Aedeagal length ranges are those characteristic of *B. carambolae* (2.34 to 2.68 mm) and *B. papayae* (2.66 to 3.34 mm) (Iwahashi, 1999). [c] Fruit fly identification followed the Centre for Agricultural Bioscience International key (White and Hancock, 1997), with further identification by measuring the length of the aedeagus and observing the dorsal abdominal pattern according to the method of Iwahashi (1999).

Conclusion

Essential oils of *M. bracteata* and *Ocimum* sp. have good potential as fruit fly attractants for controlling fruits in guava and star fruit orchards because of their ability to attract *B. carambolae* and *B. papayae*, which are major pests on guava and star fruit. Distillation water and mash extracts were effective for only about 6 days, these extracts can be used as alternatives when essential oils of *M. bracteata* and *Ocimum* sp. are not available.

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